

EXHIBIT A

Version with Markings to Show Changes

16. (Twice Amended) A method for identifying a DNA sequence which encodes a molecule or molecules which directly or indirectly modulate the interaction between at least a first and second molecule, comprising:

introducing into a host cell containing interacting molecules which generate or repress a detectable signal or growth of the cell, genomic DNA or clones of a DNA library generated from nucleic acid obtained from a mixed population of organisms and measuring the interaction of a first molecule and a second molecule in the presence of a third molecule encoded by the library or the genomic DNA or produced as a result of expression of one or more products encoded by the library or the genomic DNA, wherein interaction of the first and the second molecules in the absence of the third molecule produces a detectable signal or growth of the cell;

comparing the signal or growth of the cell in the presence and absence of the genomic DNA or library, wherein a difference between the [response] signal or growth is indicative of the presence of a molecule that modulates interaction between the first and second molecules; and

identifying a clone or DNA sequence which encodes a molecule or molecules which directly or indirectly modulates the interaction between the first and second molecules.

22. (Amended) The method of claim 16, wherein the detectable signal is encoded by a gene present in [a] the host cell.

23. (Amended) The method of claim 22, wherein the host cell further comprises a first recombinant gene encoding the first molecule, a second recombinant gene encoding the second molecule, or a third recombinant gene encoding the third molecule, [wherein the first, second or third gene are expressed in the host cell].

24. (Amended) The method of claim 23, wherein the host cell contains both the first gene and the second gene and each gene is expressed.

25. (Amended) The method of claim 23, wherein the host cell contains the first, second and third genes and each gene is expressed.

36. (Amended) A method for identifying a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting in a cell a first molecule with a second molecule in the presence of a third molecule [derived from a] encoded by a nucleic acid sequence from a library made from a mixed population of organisms or in the presence of [the] a library or genomic DNA encoding the third molecule,

wherein association of the first and second molecules in the absence of the third molecule results in the absence or presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the third molecule with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of the presence of [a] the third molecule that affects the interaction between a first and second molecule.

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42. (Amended) The method of claim 36, wherein the third molecule contains a DNA binding domain and a transcriptional activation domain.
45. (Twice Amended) The method of claim 36, further comprising, prior to step (i):
obtaining the mixed population of organisms from an environmental sample [containing a mixed population of organisms]; and
enriching the sample for prokaryotic organisms, thereby creating an enriched environmental sample, wherein said sample is used to generate the library.
46. (Amended) The method of claim 45, further comprising producing a normalized library, comprising :
isolating nucleic acids from said enriched environmental sample;
fractionating the isolated nucleic acids;
[melting the recovered fractions and allowing subsequent reannealing;] and
amplifying any single-stranded nucleic acids present in the sample.

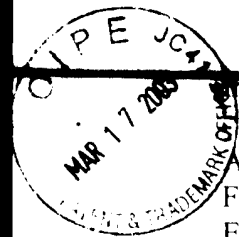
47. (Amended) The method of claim 46, further comprising generating an expression library, comprising:
inserting the amplified and isolated nucleic acids into an expression vector.

48. (Twice Amended) A method for [identifying] screening for the presence of a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting in a cell a first molecule with a second molecule wherein at least one of the first or second molecules is derived from a library made from a mixed population of organisms, wherein association of the first and second molecules in the presence of a third molecule results in the presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the third molecule and the first and second molecules with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of a first and second molecule that interact and a third molecule that affects the interaction between the first and second molecules, thereby [and] identifying the presence of a [third] molecule that affects the interaction of the first and second molecule.

49. (New) The method of claim 48, wherein a nucleic acid sequence encoding the third molecule is determined.



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16. (Twice Amended) A method for identifying a DNA sequence which encodes a molecule or molecules which directly or indirectly modulate the interaction between at least a first and second molecule, comprising:

introducing into a host cell containing interacting molecules which generate or repress a detectable signal or growth of the cell, genomic DNA or clones of a DNA library generated from nucleic acid obtained from a mixed population of organisms and measuring the interaction of a first molecule and a second molecule in the presence of a third molecule encoded by the library or the genomic DNA or produced as a result of expression of one or more products encoded by the library or the genomic DNA, wherein interaction of the first and the second molecules in the absence of the third molecule produces a detectable signal or growth of the cell;

comparing the signal or growth of the cell in the presence and absence of the genomic DNA or library, wherein a difference between the signal or growth is indicative of the presence of a molecule that modulates interaction between the first and second molecules; and

identifying a clone or DNA sequence which encodes a molecule or molecules which directly or indirectly modulates the interaction between the first and second molecules.

17. The method of claim 16, wherein at least one of the interacting molecules contains a DNA-binding moiety and at least one of the interacting molecules contains a

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18. The method of claim 17, wherein the DNA-binding moiety and the transcriptional activation moiety are derived from a single transcriptional activator.

19. The method of claim 17, wherein the DNA-binding moiety and the transcriptional activation moiety are derived from different proteins.

20. The method of claim 16, wherein the detectable signal is produced from a gene encoding a protein selected from the group consisting of β -galactosidase, green fluorescent protein, luciferase, alkaline phosphatase and chloramphenicol acetyl transferase.

22. (Amended) The method of claim 16, wherein the detectable signal is encoded by a gene present in the host cell.

23. (Amended) The method of claim 22, wherein the host cell further comprises a first recombinant gene encoding the first molecule, a second recombinant gene encoding the second molecule, or a third recombinant gene encoding the third molecule.

24. (Amended) The method of claim 23, wherein the host cell contains both the first gene and the second gene and each gene is expressed.

25. (Amended) The method of claim 23, wherein the host cell contains the first, second and third genes and each gene is expressed.

26. The method of claim 25, wherein the host cell is cultured under conditions that allows for expression of the genes.

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27. The method of claim 16, wherein the library is derived from an environmental sample.

28. The method of claim 23, wherein the third gene is derived from an environmental library.

29. The method of claim 16 or 28, wherein the environmental library is derived from an environmental sample comprising uncultured microorganisms.

30. The method of claim 29, wherein uncultured microorganisms comprise a mixture of terrestrial microorganisms, a mixture of marine microorganisms, or a mixture of terrestrial and marine microorganisms.

31. The method of claim 29, wherein the uncultured microorganisms are extremophiles.

32. The method of claim 31, wherein the extremophiles are selected from the group consisting of thermophiles, hyperthermophiles, psychrophiles, and psychrotrophs.

33. The method of claim 16 or 23, wherein the library is created by obtaining an environmental sample, enriching the environmental sample for eukaryotic organisms and selecting against prokaryotic organisms, isolating nucleic acids from the enriched sample, fractionating the nucleic acids, and cloning the isolated nucleic acids into a vector.

34. The method of claim 33, wherein the nucleic acids are amplified prior to

35. The method of claim 33, wherein the vector is an expression vector.

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36. (Amended) A method for identifying a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting in a cell a first molecule with a second molecule in the presence of a third molecule encoded by a nucleic acid sequence from a library made from a mixed population of organisms or in the presence of a library or genomic DNA encoding the third molecule,

wherein association of the first and second molecules in the absence of the third molecule results in the absence or presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the third molecule with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of the presence of the third molecule that affects the interaction between a first and second molecule.

37. The method of claim 36, wherein the detectable response is the expression or repression of a detectable gene.

38. The method of claim 37, further comprising, prior to (i):

providing a prokaryotic host cell containing the detectable gene; and
providing a first gene expressed in the host cell, the first gene encoding the first molecule.

39. The method of claim 38, further comprising, prior to (i):

providing a second gene expressed in the host cell, the second gene

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40. The method of claim 39, further comprising, prior to (i):
providing a third gene expressed in the host cell, the third gene encoding
the third molecule.

41. The method of claim 40, further comprising, prior to (i):
introducing said first, second and third genes into the host cell; and
allowing expression of the genes.

42. (Amended) The method of claim 36, wherein the third molecule contains a
DNA binding domain and a transcriptional activation domain.

43. The method of claim 36, wherein the interaction between the first and second
molecules forms a transcriptional repressor.

44. The method of claim 36, wherein the third gene is derived from an
environmental library.

45. (Amended) The method of claim 36, further comprising, prior to step (i):
obtaining an environmental sample containing a mixed population of
organisms; and
enriching the sample for prokaryotic organisms, thereby creating an
enriched environmental sample.

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46. (Amended) The method of claim 45, further comprising producing a normalized library, comprising :

- isolating nucleic acids from said enriched environmental sample;
- fractionating the isolated nucleic acids;
- and
- amplifying any single-stranded nucleic acids present in the sample.

47. (Amended) The method of claim 46, further comprising generating an expression library, comprising:

- inserting the amplified and isolated nucleic acids into an expression vector.

48. (Twice Amended) A method for screening for the presence of a molecule that affects the interaction between a first and second molecule, comprising:

- (i) contacting in a cell a first molecule with a second molecule wherein at least one of the first or second molecules is derived from a library made from a mixed population of organisms, wherein association of the first and second molecules in the presence of a third molecule results in the presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

- (ii) comparing the detectable response in the presence of the third molecule and the first and second molecules with the detectable response in the absence of the third molecule, wherein a difference in response is indicative of a first and second molecule that interact and a third molecule that affects the interaction between the first and second molecules, thereby identifying the presence of a molecule that affects the interaction of the first and second molecule.

49. (New) The method of claim 48, wherein a nucleic acid sequence encoding the third